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Chemical Antagonism of Red-Winged Blackbird Habituation to Fright-Producing Auditory Stimuli

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We studied the feasibility of improving the efficacy of fright-producing auditory stimuli as wild bird repellents by chemically antagonizing habituation to the stimulus. Red-winged blackbirds (*Agelaius phoeniceus*) were fed mealworms containing a 10 mg/kg dose of D-amphetamine sulfate and exposed to a prerecorded blackbird distress call. Heart rate (HR) and behavioral activity responses (BAR) of these birds were measured and compared to the responses of untreated birds. After an initial strong response, the HR and BAR of control birds diminished despite repeated applications of the distress call until, after 30 applications, they were near the baseline values. The HR and BAR of birds treated with D-amphetamine sulfate remained at elevated levels throughout. The treated birds' responses demonstrated that D-amphetamine sulfate was effective as an habituation antagonist. The results suggest that a nonlethal level of a central nervous system stimulant could be used to antagonize habituation of wild birds to auditory repellents, thereby enhancing their effectiveness in control programs.

INTRODUCTION

Wild birds are generally responsive to auditory stimuli of biological origin, especially alarm and distress sounds (1–7). Such stimuli have been widely used to repel nuisance birds with various degrees of success (1, 2, 5, 6). The use of auditory stimuli to repel birds is appealing because it is harmless and humane to both target and nontarget species and the time and expense of registering lethal control agents is avoided. The increasing concern over humane treatment of animals and the high cost of chemical registration warrant exploratory research to improve the efficacy of auditory bird repellents.

The major limitation to effective use of auditory stimuli to repel birds is rapid habituation. Delaying habituation would improve the usefulness of auditory stimuli for control programs; therefore, we explored the possibility of chemically antagonizing habituation to repeated applications of fright-inducing distress sounds. The measurement of physiological and behavioral responses was done by recording heart rate (HR)[1] and behavioral activity responses (BAR) of red-winged blackbirds (RWBB) (*Agelaius phoeniceus*) to prerecorded distress sounds alone and in combination with sublethal dosages of a central nervous system (CNS) stimulant, D-amphetamine sulfate. We used D-amphetamine sulfate because of its ability to potentiate startle response to an auditory stimulus in mice and rats (8, 9) and antagonize skin conductance habituation response to a light stimulus in mice (10). To our knowledge there are no reports of its use as a habituation antagonist in birds. We monitored HR by means of biotelemetry in order to avoid frightening the birds by the measuring process.

METHODS

The experiment was conducted with 20 male RWBB that had been maintained in an indoor aviary for 1 to 3 months. Each bird selected for testing was examined to assure

[1] Abbreviations used: HR, heart rate; BAR, behavioral activity responses; RWBB, red-winged blackbirds; CNS, central nervous system; dB, decibels.

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good health. This included checking body weight, breast muscle size in relation to sternum prominence, and overall alertness and vigor of the bird. Birds were then caged and maintained on a mixture of small grains supplemented with mealworms. Ten birds were randomly assigned to a control group (auditory stimuli plus untreated worm) and 10 to a treatment group (auditory stimuli plus worm treated with D-amphetamine sulfate). They were maintained under ambient window light supplemented with room light on an ambient cycle.

The birds were deprived of food about 4 hr before placement in the test chamber for observation. While a bird was in the chamber for approximately 30 to 60 min daily, we offered each bird live mealworms from a remotely controlled manually operated dispensing apparatus. After they adjusted to the chamber and to eating the mealworms, the birds were instrumented with sensors and a miniature back-mounted radiotransmitter to telemeter and record HR as a second order function of the electrocardiogram (7, 11, 12). Birds were permitted to adjust to the transmitter, sensors, and behavior chamber before initiating tests. The adjustment period lasted until baseline HR stabilized at 400 or lower. Heart beats were counted continuously and total beats for each minute were printed during pretreatment and experimental test periods.

The test chamber isolated the bird from outside stimuli and was equipped with a speaker to introduce prerecorded sounds and closed circuit television camera to observe behavioral performance. The speaker was connected to a tape cartridge handler and amplifier to manually deliver 10 sec of a prerecorded yellow-headed blackbird (*Xanthcephalus xanthcephalus*) distress call at 78 decibels (dB). We had previously selected the recording as the most effective distress call among several we had recorded. The selection was based on HR response of RWBB where we used a technique similar to that described in a previous report (7).

The D-amphetamine sulfate was formulated into a gel, placed in a microsyringe, and then injected into live mealworms with a 25-gauge needle so as to provide 10 mg/kg/bird. The LD_{50} of D-amphetamine sulfate for red-winged blackbirds is 56.0 mg/kg (13). The gel was a mixture of 1.25 g Syloid 244[2,3] (*amorphus silicon dioxide*), 5 ml of deionized water, and in the treated formula, 325 mg of D-amphetamine sulfate. The gel was used instead of a liquid to prevent loss of dose by oozing at the injection point on the mealworm. Two untreated worms and one treated worm, gel only in control group, were placed in a dispenser. Manual activation of the dispenser injected the untreated worms into a food cup in the presence of the test bird. After eating the two untreated worms, the bird was offered the treated worm. Baseline-pretreated HR was recorded for 1 min immediately prior to injection of mealworms into the food cup.

The 10 birds in the treatment group were each given a mealworm containing D-amphetamine sulfate. Beginning 10 min after birds voluntarily ate the treated mealworm, they were subjected to 10 sec of auditory stimuli 30 times, at 3-min intervals. This resulted in a total of 90 min that a bird was tested for response to the distress call.

Birds were observed on closed-circuit television and were assigned a BAR rating of 1 to 5 (see Table 1). HR and BAR were recorded continuously and printed at 1-min intervals. The first minute of data recorded after the auditory stimulus began was used to represent HR and BAR values. We believe that this period of bird response best depicts the birds' reaction to the auditory stimulus. The 10 birds in the control group were handled the same way as the treatment group with the exception that their mealworms contained no D-amphetamine sulfate.

HR and BAR of treated birds versus con-

[2] Reference to trade names does not imply endorsement of commercial products by the federal government or any of its agencies.

[3] Davidson Chemical Division, WR Grace & Co., Baltimore, MD.

TABLE 1
Behavioral Activity Rating of Red-Winged Blackbirds

Rating	Description of activity
1	Bird sitting on perch or floor, minimal movement.
2	Bird shifts location several centimeters, shows alertness by frequent head movements.
3	Bird changes positions by turning around or moving to another location in behavioral chamber.
4	Frequent movement from perch to floor and floor to perch or from corner to corner, etc.; stereotyped activity pattern.
5	Rapid movement that includes frequent wing flapping for 30 sec following stimulus onset.

trol birds were analyzed using ANOVA and regression techniques. The study design included a provision to establish the HR at which the bird would be considered habituated to the stimulus. Preliminary testing with 10 birds indicated that HR less than 400 could be considered habituated. This was subsequently confirmed as a valid cutoff point when the pretreatment HR values of the 20 birds in the study were determined to average 377. The birds were tested with this in mind; any bird that calmed to a HR below 400 was not subject to additional auditory stimuli. Subsequent examination of the data revealed that a response curve depicting the 30 response periods would best display the results. As a consequence, an estimated HR value of 400 was inserted for each missing value for those birds removed from the study before the 30th auditory stimulus. The resultant response curve is not quite as steep as it would have been with all the values present since missing values replaced with a 400 HR normally would trend lower than 400. The BAR missing values were replaced with a value of 1.

RESULTS AND DISCUSSION

The methodology in this study facilitated data retrieval from birds that were essentially unrestrained and free of human interference. Most of the birds readily adapted to experimental conditions as evidenced by the stabilization of HR and the performance of activities such as perching, preening, and eating mealworms after a few 30- to 60-min sessions in the test chamber.

Pretreatment mean baseline HR for control and treated birds were 379 ± 13 SE and 374 ± 13 SE, respectively; this is not a significant difference ($P = 0.769$). The overall mean was 377 ± 9 SE. Figure 1 depicts the HR response of control and treated birds to the auditory stimulus. The response patterns are markedly different. With the onset of the first auditory stimulus, HR of control birds accelerated to a mean peak level of 679 ± 22 SE. During the 2 and 3 min following each stimulus, the HR decelerated and when the stimulus was repeated, HR accelerated rapidly again but did not attain the previous peak level. The HR response curve of the control birds declined logarithmically to 414 ± 12 SE after the 30th sound stimulus. The form of the response (Fig. 1) is described by the equation $Y = 699 - 80.59 \ln X$, where Y = HR and X = periods (sound stimulus applications). The control HR equation provides a good fit to the data ($P < 0.001$, $r^2 = 0.90$).

The HR response curve for treated birds is a fifth degree polynomial equation, $Y = 558 + 0.648X - 2.549X^2 + 0.306X^3 - 0.0125X^4 + 0.000168X^5$, where Y = heart rate and X = periods (sound stimulus applications). The treated HR curve is not as good a fit to the data as the control curve but is still adequate ($P < 0.001$, $r^2 = 0.74$).

The two peak HR responses of treated birds occurred at 554 ± 25 SE and 548 ± 25 SE. The first occurred after the initial exposure to the stimulus and the second an hour later after being subjected to 20 stimulus applications. It is obvious that the control and treated HR response curves each represent a different type of reaction to the stimuli. The control birds show a typical

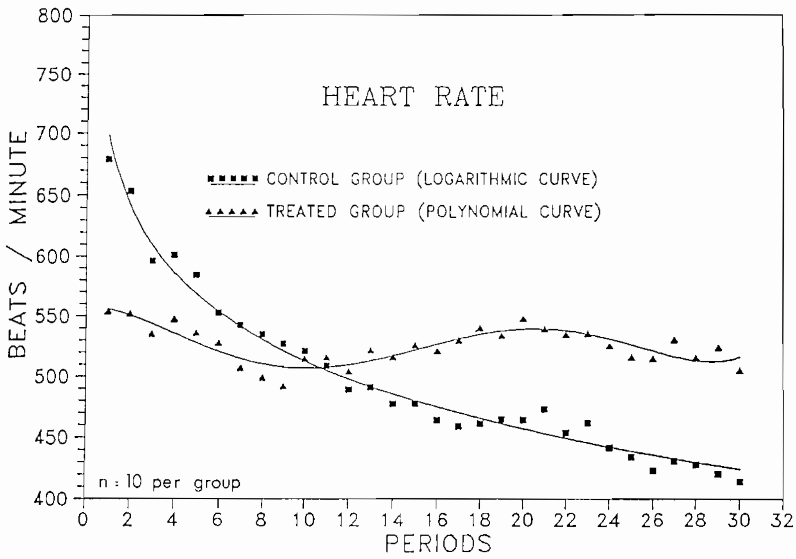

FIG. 1. *Heart rate of control and treated groups of red-winged blackbirds exposed to a fright-producing auditory stimulus. Each bird in the treated group received a 10 mg/kg oral dose of* D-*amphetamine sulfate.*

response to an auditory stimulus; initial fright becomes a diminishing reaction that approaches pretreatment values. The response of treated birds showed only a slight declination of HR during the 30 stimulus periods. The fact that HR values of treated birds did not reach levels as high as the control birds during the first 10 periods can be explained. In animals orally dosed with amphetamine, both systolic and diastolic blood pressure are raised; the arterial baroreceptors sense the increased pressure and respond as part of a reflex designed to slow the heart (14).

The initial reaction of control birds was very pronounced and was comparable to previous reports (7, 11) about the response of wild starlings (*Sturnus vulgaris*) to distress sounds. The HR of some accelerated to over 700, which is approximately 100% above baseline HR and near their physiological response capability. Nevertheless, if an habituation point of HR 500 is selected, the average values for all control birds show habituation after 12 consecutive stimulus applications (Fig. 1) and continued calming until the end of the 30th period. The average values for all birds dosed with D-amphetamine sulfate depict a continuing reaction to the stimulus 30 times without habituation; they were still in an excitable condition after the 30th sound application. When looking at the response of each individual bird, 4 of the 10 chemically dosed birds did not habituate to the sound stimulus during the 90-min test period; conversely, all control birds habituated before the 15th sound applications, 42 min into the study.

The BAR curves are shown in Fig. 2. The control birds' BAR curve declined logarithmically as expressed by the equation $Y = 2.537 - 0.303 \ln X$, where Y = BAR and X = periods (sound stimulus applications). This curve is a good fit to the data ($P < 0.001$, $r^2 = 0.62$). The curve for the treated group is a fifth degree polynomial equation, $Y = 3.614 - 0.195X + 0.0189X^2 - 0.000550X^3 - 0.00000129X^4 + 0.000000178X^5$, where Y = BAR and X = periods (sound stimulus applications). This curve also provides a good fit to the data ($P = 0.004$, $r^2 = 0.52$).

The behavioral activity (Fig. 2) for both

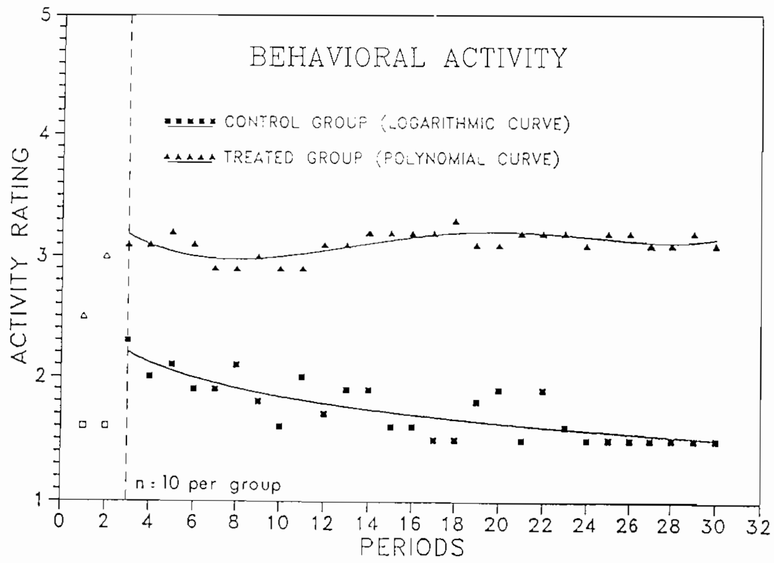

FIG. 2. *Behavioral activity of control and treated groups of red-winged blackbirds exposed to a fright-producing auditory stimulus. Each bird in the treated group received a 10 mg/kg oral dose of D-amphetamine sulfate.*

control and treated birds is illustrated by responses beginning with the third sound application; data from the first two applications indicated that the birds were not yet fully reacting and they were not included in the analysis. The birds behavioral response during the first two sound applications appeared to be a condition of high alertness, as detected by the HR, yet little movement as if the birds were confused and not certain how to respond. The treated birds' initial BAR values averaged 3.2 and remained near a level of 3 for the entire 30 sound applications. Six treated birds scored a BAR of 5 at various times during the 90-min test; control birds never exceeded a BAR of 4. The response curve of the control birds starts at a BAR of 2.2 and slowly declines to a 1.5 rating where it remains from the 24th thru the 30th sound application.

The data presented demonstrate that the D-amphetamine sulfate was effective as a habituation antagonist. To our knowledge, this is the first report on the use of a chemical to delay or inhibit onset of habituation of wild birds to auditory stimuli. Conceptually, the results suggest that CNS stimulants could be used to antagonize habituation of wild birds to auditory repellents, thereby enhancing their effectiveness in control programs.

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